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Research Article

ENHANCED TOTAL PHENOLIC CONTENT EXTRACTION FROM CUCUMIS MELO L. (KULTIK) KERNEL BY DEEP EUTECTIC SOLVENT (DES)

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Abstract: *The kernel of Cucumis melo L is a by-product produced from the melon production process. The phenolic compounds could be considered as a potential bioactive source for industrial applications. Therefore, the extraction of these compounds as much as possible will decrease valuable waste and could lead to producing value-added products. In the first part of this study, a comparison of the effect of DESs and conventional solvents on total phenolic content (TPC) extraction yield was performed. Some DESs had significantly better extraction yields than conventional solvents. Therefore, optimization of extraction conditions was performed by single factor experiment. Optimized parameters are molar ratio, type of HBA (hydrogen bond acceptor), the addition of water content, extraction time, and extraction temperature. From the results obtained, all these parameters were found to have an impact on TPC extraction yield. Also, it is noteworthy that the extraction yield using some selected parameters was on decreased after a certain extent. The best extraction parameter for Cucumis melo L. was found to be choline chloride as HBA, 1:4 molar ratio, 30% water addition, 50^0 extraction temperature, and 30 min extraction time. This result confirms that kernel of Cucumis melo L. is a valuable ingredient due to its bioactive content, DESs could be a good alternative to conventional solvents and the industrial applications of DESs could be possible.*

Keywords: *Chemistry, Eutectic, Extraction, Green, Kultik.*

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1. Introduction

Cucumis melo L. (Kultik), one of the melon varieties, has been widely cultivated and consumed in Turkiye. It has a unique kernel, which is shell-less. Even though the fruit of *Cucumis melo* L. is regularly consumed, the kernel was deemed as food waste in Turkiye. Therefore, it has no economic value, leading the kernel of *Cucumis melo* L. to pose serious environmental problems. However, it has many bioactive compounds including valuable phenols [3]. In previous studies, consuming ng kernel of *Cucumis melo* L. was advised due to its bioactive content and was used as an ingredient to produce high-value food products [9,10]. Moreover, utilizing kernel of *Cucumis melo* L. as a source for high-value products is compatible with green extraction technology.

Extraction conditions, including time and temperature, have a major effect to recover phenolic contents in plant materials. Unfortunately, there is no standard method owing to the diverse physicochemical properties of phenolic compounds in plant materials for extraction. Thus, the extraction of each sample must be optimized by specified advantageous conditions for the targeted compound to acquire maximum yield [14]. It is the first time as far as the author knows that the total phenolic content of *Cucumis melo* L. cultivated in Tunceli has been determined.

Conventional solvents were widely used in previous studies to extract phenolic compounds in plant materials [2,25,31]. Differentiation among conventional solvents' properties influences extractable total phenolic content due to secondary metabolites, which show changeable solubility and mass transfer. Methanol, with water as a co-solvent, usually gave the highest TPC in plant materials [14]. However, these solvents have disturbed disadvantages, such as high toxicity and irreversible damage to nature and human life. A ceaseless demand rises to find a green solvent, deemed as an alternative to conventional solvents [5]. Many recent studies showed that deep eutectic solvents (DES) could be a good option for conventional solvents [6,21,30,34]. DESs are mainly formed by mixing hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD). But more components could be added to the formation of DESs. The extraction efficiency of DESs could show differences according to many factors, including types and molar ratios of DES-forming components [21].

This study is targeted to help future studies investigate possible ways solutions for environmental problems caused by food waste and conventional solvents. Therefore, the first objective of this study is to determine the total phenolic content (TPC) of *Cucumis melo* L. by using a conventional solvent (50% Methanol + 50% Water). The second objective is the comparison of TPC extraction efficiency between conventional solvents and DESs to identify whether DES could replace conventional solvents in extraction. The last objective optimizes extraction conditions to achieve maximum TPC yield by DES. Optimized extraction conditions were molar ratio, type of HBA, extraction temperature, extraction time, and co-solvent, namely water, addition.

2. 2. Materials and Methods

2.1. Materials

2.1.1 Sample Preparation Process

Cucumis melo L. kernel used in this study was bought from local producers in Tunceli province, Turkiye during two consecutive years 2021 and 2022 to identify the effect of year variance. After the samples were collected from 5 different producers, they were taken to Munzur University Food Engineering laboratory. The samples were kept at 20^0C in the laboratory.

The sample (at least 250 g) was weighed, then blended by using a kitchen blender (Fisher Scientific, Model 8010ES). All the powder in the blender was mixed. The mixed sample was used for the analyses. The procedure was separately performed for the samples harvested in different years.

2.1.2 Solvent Preparation Process

DESs were prepared according to [16] with some modifications. The components were weighted with various molar ratios in a breaker shortly after they were dried. The breaker was kept in a hot air oven until a homogeneous transparent liquid was formed. The mixture was cooled down to room temperature, then kept at room temperature to store in sealed vessels until their utilization. DES varieties used in this study were displayed in Table 1.

A conventional solvent (50% Methanol + 50% water) was selected for extraction as a comparison to DESs according to the study, which found that phenolic compounds in *Crataegus orientalis* were better extracted with 50% Methanol + 50% water than that with 100% any of conventional solvents [14].

Table 1. Type of DES used in this study.

Type	Combination	Molar Ratio
DES-1	Zinc Chloride: Ethylene Glycerol	1:1
DES-2		1:2
DES-3		1:3
DES-4		1:4
DES-5		1:5
DES-6		1:6
DES-7		1:7
DES-8		1:8
DES-9		1:9
DES-10		1:10
DES-11	Choline Chloride: Ethylene Glycerol	1:4
DES-12	Glucose: Ethylene Glycerol	1:4

2.2. Methods

2.2.1 Total phenolic content

Determination of total phenolic content in the extracts was performed using Singleton's method with some modifications [14]. Briefly, 1 mL of diluted extract was mixed with 5 mL of folin- ciocalteu solution (0.2 N) and vortexed. After the prepared solution was kept dark for 5 minutes, 8 ml of sodium carbonate solution (7.5%) was added to the mix, then the mix was incubated at room temperature for 2 hours. Thereafter, the absorbance values of the samples were measured at 765 nm by UV-Vis spectroscopy. The result was expressed as mg Gallic acid equivalent (GAE) g^{-1} dw of the sample. The standard curve of gallic acid was conducted with various concentrations for each solvent variety used in this study with good linearity (r^2>0.99). For each sample, the Folin-Ciocalteu assay was performed in triplicate.

2.2.2 Determination of optimal extraction conditions

Many factors could affect TPC extraction, but, in this study, three parameters were decided to use by single factor experiment. The effect of the parameters was evaluated according to the statistical analysis result.

2.2.2.1 Evaluation of optimal molar ratio and HBA type

Various molar ratios, including 1:1 to 1:10 HBA-HBD, were utilized. Thereafter, the effect of HBA type on TPC extraction yield was evaluated by using DESs with the best molar ratios (1:4) determined in the earlier part.

2.2.2.2 Evaluation of optimal water content addition to DES

Different water content, ranging from 10% to 50%, was added to determine the effect of water addition to DES. Namely, 10 mL water was added to 100 mL DES to give a 10% water addition.

2.2.2.3 Evaluation of optimal extraction temperature and time

The extraction was performed with DES (70% DES (1:4 molar ratio of ChCI:EtGl) + 30% water), which was determined as the best molar ratio to extract phenolic compounds in the earlier part of this study. Different extraction times (0, 15, 30, 45, and 60 min) were used to compare the effect of extraction time on extraction efficiency at 30^0C, which was chosen by the author according to [6]. Then, the effect of extraction temperature on TPC extraction was evaluated by using various temperatures (30^0C, 50^0C, 60^0C, and 80^0C) in 30 min, which was determined to be optimal in the earlier part of this study.

Statistical Analysis

All the analyses were performed in triplicate. Each data was subjected to a homogeneity test (Shapiro-Wilk). The results of the test showed that all the data was distributed homogenously; thus, parametric methods were determined to use for all the data found in analyses. Therefore, the data were subjected to analysis of variance (ANOVA) and independent t-test using Statistical Package for Social Science (SPSS) version 29.0 software. Means were separated by using Duncan Multiple Range Test. The level of significance of differences between treatments was determined at $p<0.05$.

3. Results and discussion

Various molar ratios of ZnCI: ethylene glycerol were synthesized to identify the effect of molar ratio on TPC extraction yield. In the first part, 1:1 to 1:10 molar ratios of ZnCI: EtGI were examined, thereafter ZnCI, ChCI, and glucose were used as HBA, and ethylene glycerol was the HBD for understanding the effect of HBA. The increasing HBD molar ratio in the solvent rose the extraction efficiency of phenolic content in the sample to a 1:4 molar ratio, with the highest extraction efficiency of phenolic content being found at a 1:4 molar ratio. A steady decrease in phenolic content extraction was screened from a 1:4 molar ratio to a 1:10 molar ratio of ZnCI: EtGl.

The maximum extraction yield of TPC was determined at a 1:4 molar ratio, 150.24 mg GAE g^{-1} dw in 2021 and 148.21 mg GAE g^{-1} dw in 2022 by ZnCI: Ethylene Glycerol (EtGl). A dramatic and steady decrease was clearly monitored on extracted TPC after the 1:5 molar ratio, with a steady increase being screened from 1:1 to 1:4 molar ratio.

Table 2. Effect of molar ratio parameter on TPC in extracts from *Cucumis melo* L. by Zinc chloride: ethylene glycerol.

Type	Total Phenolic Content (mg GAE g^{-1} dry weight of the sample) 2021	2022	T-test
Conventional Solvent	79.22 ± 9.72e	86.96 ± 5.14e	0.290
DES-1	63.86 ± 3.27d; D	71.71 ± 1.43d; C	0.010
DES-2	91.38 ± 4.68f; E	96.04 ±3.79e; D	0.189
DES-3	112.69 ± 7.82g; F	108.26 ± 5.44f; E	0.466
DES-4	150.33 ± 7.24j; H	148.62 ± 9.05h; G	0.844
DES-5	128.16 ± 5.54h; G	119.93 ± 5.19g; F	0.064
DES-6	27.40 ± 2.17c; C	26.14 ± 1.03b, c; B	0.388
DES-7	23.16 ± 2.62b, c; B,C	20.82 ± 1.78a, b; A,B	0.299
DES-8	15.73 ± 1.52 a, b; A, B	15.22 ± 0.69a, b; A	0.626
DES-9	12.17 ± 1.91a, b; A	12.44 ± 2.48a; A	0.885
DES-10	10.62 ± 0.60a; A	11.92 ± 0.79a; A	0.086

Note: Different letters in the same column show a significant difference ($p< 0.05$).
Lower case: it was used for the solvents including conversational solvents and different molar ratios of ZnCI: EtGI.
Upper case: it was used for ANOVA analysis of DESs having the same HBA with different molar ratios

It is noticeable that some DESs significantly better extracted TPC than the conventional solvent. A 1:4 molar ratio was found the most favorable for the best extraction yield. Significant differences among different molar ratios of ZnCI: EtGl were found, with year variance causing no significant influence on TPC extraction yield, except for the 1:1 molar ratio. The exception might be related to the difference in the solvent's polarity, which could affect the molar ratio of DES [1,15]. Ozturk et. al. [28]

stated that the extraction yield of TPC from orange peel waste changed with regard to the polarity, which showed a difference according to the molar ratio of DESs. This result is in according with the study of Li et. al. [23], which observed that a 1:4 molar ratio is the best for TPC extraction yield for amino acid-based DESs. Moreover, some DESs of ZnCI: EtGl with different molar ratios showed significantly better extraction yield than conventional solvent, which was expressed to be the best conventional solvent for extraction from plant materials [14]. This result means that DESs could have a chance to take the place of conventional solvents in TPC extraction from plant materials. It is a need to state that, as far as the author's experience, using 1:1 and 1:2 molar ratios of HBA: HBD was extremely difficult for TPC extraction due to high viscosity. Even though a constant acceleration on TPC was observed from 1:1 to 1:4 molar ratio, a catastrophic decrease screened on TPC extraction yield by DES after 1:5 molar ratio. This result could be related to low viscosity, which was reported not to be appropriate to extract target compounds [24].

Results of previous studies showed differentiation for the best molar ratio of DES in TPC extraction. Some studies, which are in according with this study, stated that 1:4 molar ratio of HBD:HBA gave the highest extraction efficiency of phenolic content in plant materials [21,30]. However, Luo et. al. [25] demonstrated that the highest phenolic content was found in green tea (*camellia sinesis*) extracted by the combination of ultrasonic-assisted extraction and a 1:2 molar ratio of choline chloride: glycerol. Chen et. al. [12] reported that extraction yield for the selected compound from Radix *salviae miltiorrhizae* steadily decreased as the molar ratio of ChCI used as HBA was on the decrease, and that 1:3 molar ratio of ChCI: glycerol had better extraction efficiency than 1:4 molar ratio. The differences in the results of the studies could be due to many factors, including the part of plant materials, the plant growing conditions, and even the extraction process [31,38]. In addition, the differences in DES extraction yield may happen due to structural differences, which could be easily modified by the choice of DES-forming components shaping hydrogen bond constitution [37].

To evaluate the effect of HBA type on TPC extraction yield, choline chloride (ChCI) and glucose were used in comparison to ZnCI. A 1:4 molar ratio was determined to use for comparison of HBA type due to the result in the early part of this study. It was found that HBA type in DES could significantly affect TPC extraction yield in plant material, with ChCI: EtGI significantly having higher extraction yield when glucose: EtGI significantly having the lowest extraction efficiency, $p<0.01$. ChCI as HBA was also observed as the best for TPC extraction by many previous studies [2,8,12,36]. Thus, it is necessary to express that even though many studies just focused on the type of HBD for better extraction yield and reported that type of HBD mainly affects extraction yield [7,22,29,33], this study found that the type of HBA also has a precise effect on extraction yield.

Table 3. Effect of HBA (Hydrogen bond acceptor) parameter on TPC in extract from *Cucumis melo* L.

Type	**Total Phenolic Content (mg GAE g^{-1} dry weight of the sample)** 2021	2022	**T-test**
DES - 4	150.33 ± 7.24b	148.62 ± 9.05b	0.844
DES-11	163.34 ± 12.73c	162.76 ± 6.00c	0.961
DES-12	27.40 ± 6.37a	32.27 ± 4.50a	0.341

The extraction yield of phenolic components available in foods could be enhanced depending on extraction conditions and suitable co-solvent usage [32]. Many studies reported that conventional solvents with a co-solvent were more effective in TPC extraction [4,35]. The addition of co-solvent to DES was mentioned to have the potential to increase extraction efficiency in plant materials due to better solvent penetration into the sample [40]. Thus, in order to determine the effect of adding a co-solvent, namely water, to DES on TPC extraction, a 1:4 molar ratio of ChCI: EtGl was decided to use due to that it gave the highest phenolic content from *Cucumis melo* L kernel in the earlier part of this study. TPC extraction was performed with DES containing different percentages of water ranging from 10% to 50%. The yields of TPC extracted by any combination of DES and water from *Cucumis melo* L kernel were significantly higher than DES without any water addition. It could be due to that adding water to DES could reduce viscosity, and lower viscosity led to higher mass transfer [19]. The same result was also found in previous studies [27,34,37,39]. Moreover, there wasn't any significant difference among DESs including various water content, with 70% DES + 30% water having the highest extraction yield than any combination of DES and water. Even though it wasn't significant (p>0.05), higher than 30% water addition to DES led to lower extraction yield in this study. The same result was also observed by previous studies [12,20,27,39,42]. Chen et. al. [12] enhanced the extraction efficiency of the selected compounds by adding water to ChCI-1,2-Propanediol up to a certain extent. Wu et. al. [36] stated that adding higher than 40% water to DES led to lower TPC extraction yield in *Polygonum aviculare* leaves. New et. al. [27] emphasized that maximum extraction efficiency of lignin was found for DES (ChCI-Urea) including 20% water content. The increase in extraction efficiency as water content rises to a certain extent might be owing to better solvent penetration and diffusion of the solute targeted in the sample [42]. The lower extraction yield after a certain extent could be related to the interaction between co-solvent and CO_2 [13] or destroying effect of co-solvent concentration on the hydrogen bonds between DES-forming components [17]. In addition, Chen et. al. [12] indicated that as the water content in DES was on the increase, better extraction yield was observed for hydrophilic compounds up to a certain extent (60%-80% water content, depending on the targeted component) when water extraction yield was monitored for hydrophobic compounds. The differences among the studies reveal that extraction of TPC in the sample needs an individual approach.

Table 4. Effects of extraction parameters on TPC in extracts from *Cucumis melo* L.

Factors	**Total Phenolic Content (mg GAE g^{-1} dry weight of the sample)**	
Water Content*	2021	2022
10%	245.46 ± 8.22a	221.67 ± 41.11a
20%	253.86 ± 14.67a	236.47 ± 21.60a, b
30%	282.48 ± 20.40b	277.59 ± 15.07b
40%	265.81 ± 4.44a, b	251.69 ± 20.87a, b
50%	257.59 ± 13.71a, b	228.38 ± 24.29a, b
Extraction Time**		
0 min	282.48 ± 20.40a	277.59 ± 15.07a
15 min	311.22 ± 14.13a	286.49 ± 12.74a
30 min	398.36 ± 32.82b	379.52 ± 24.81b
45 min	399.54 ± 21.19b	381.87 ± 45.10b
60 min	417.20 ± 14.13b	392.47 ± 40.74b
Extraction Temperature***		
30^{0}C	398.36 ± 32.82a	379.52 ± 24.81a
50^{0}C	814.96 ± 12.50c	719.67 ± 12.10c
60^{0}C	567.86 ± 12.12b	504.46 ± 4.98b
80^{0}C	524.74 ± 31.81b	407.72 ± 15.22a

Note: Different letters in the same column show a significant difference (p < 0.05). *Extraction conditions: L/S ratio: 100:1 mL/mg; extraction temperature: 30°C; extraction time: 0 min; **Extraction conditions: L/S ratio: 100:1 mL/mg; Water content: 30%; temperature: 30°C; ***Extraction conditions: L/S ratio: 100:1 mL/mg; Water content: 30%; extraction time: 30 min.

Extraction time was evaluated using various times ranging from 0 minutes to 60 minutes. Extraction time was found to affect TPC extraction yield. Just after mixing DES (70% DES + 30% water) with the sample, TPC was found to be 282.48 mg g^{-1} dw for kernels harvested in 2021: and 277.59 mg g^{-1} dw for kernels harvested in 2022. Extracted TPC was on the increase as extraction time was on the increase. Extraction time is also an important parameter in TPC extraction from plant materials. Generally, shorter extraction time with the same extraction yield is demanded enhanced process applications [23]. A significant change in extraction yield according to extraction time was observed, with no significant difference being found after 30 min. This result is in according with the result of previous studies [11,26,37,42]. Even though Wu et. al. [36] didn't find any significant difference among extraction times of 30, 40, and 50 minutes, Mansinhos et. al. [26] stated that even if there was not a significant difference between 15 min and 30 min of extraction times, 60 min having significantly better TPC extraction yield. Zhou et. al. [42] also found the same result, which showed no significant difference between 15 min and 30 min of extraction time for some of the phenolic compounds from *Morus alba* L., with [26]. The difference among the studies could rise due to the extraction process, which was differently done.

Temperature is one of the key factors leading to changes in extraction yield. The effect of temperature on TPC extraction by the DES (70% DES (ChCI- ethylene glycerol, 1:4 molar ratio) + 30% Water) was investigated. To evaluate the effect of extraction temperate, temperatures ranging from 30^0 to 80^0 were performed. Extraction time was chosen as 30 min according to the early part of this study, which found that there wasn't any significant difference in TPC extraction yield after 30 min (Table 4). The highest TPC was found at 50^0 extraction temperatures. The extraction yield of TPC by DES regularly decreased as the extraction temperature rose after 50^0. Extraction temperature was determined to have an impact on extraction yield. In previous studies, it was stated that a rise in the extraction temperature within a specific range usually led to lower viscosity of DES, which enhances better mass transfer, and eventually higher analyte solubility [18]. In this study, a significant difference was determined among extraction temperatures for extraction yield of TPC, with the highest TPC being found at 50^0 extraction temperatures. It is eye-catching that the extraction yield of TPC by DES regularly decreased as the temperature rose after 50^0. The same result, the decrease in the yield after 50^0, was also demonstrated by previous studies [20,23,36,37]. However, Bildik [6] and Zhou et. al. [42] monitored the same decrease in TPC extraction of Rheum Ribes leaves by ChCI-based DESs just after 40^0. The reason why the TPC value was on the decrease as extraction temperature was on the increase after a certain extent could be due to lower mass transfer [16]. In addition, extraction conditions led to the difference in the study of [6] and [42] which used ultrasound-assisted extraction. Zhou et. al. [42], which used extraction temperatures ranging from 30^0 to 60^0, found the lowest extraction yields of phenols at 30^0. This result is in accordance with the result found in this study. High or low extraction temperatures may negatively influence the stabilization capacity of the extraction assay, which changed due to the low or high viscosity of DES, and this may lead to limited mass transfer [16].

4. Conclusions

This study found that *Cucumis melo* L kernel has high total phenolic content, which makes it an important substance for the extraction process. TPC of the sample had 79.22 mg GAE g^{-1} dry weight in 2021, and 86.96 mg GAE g^{-1} dw in 2022 by conventional solvent (50% methanol + 50% water).

Twelve DES varieties were used in this study to compare TPC extraction yield to conventional solvent. It was found that some DESs could better extract TPC in the sample than conventional solvents. This result represents an enormous opportunity for the utilization of DESs in industrial applications.

Optimization of TPC extraction conditions using DES was also investigated by single-factor experiments. The molar ratio of DES was found to have a significant effect on TPC extraction yield.

The highest TPC was found at the 1:4 molar ratio of the DES type investigated. Further, the DES-forming component as HBA was observed to change extraction yield, with ChCI having statistically the best extraction yield. Moreover, it was important to note that a higher than 1:5 molar ratio caused a dramatic reduction in the extraction yield of TPC.

Adding a co-solvent, namely water, has been reported to affect extraction yield by DES. All the combinations of DES and water showed better extraction efficiency than the DES without any addition of co-solvent. The highest total phenolic content extraction was determined for 70% DES + 30% water. TPC extraction yield was on the increase as water content was on the increase by up to 30% water addition. It is important to state that even though it was not significant, higher water content than 30% could lead to a decrease in TPC extraction yield.

Extraction temperature and time were also game-changing factors. The highest extraction yield was obtained at 50^0 with 30 min extraction time. It was important that TPC extraction yield was on the decrease as extraction temperature was on the increase after 50^0. Additionally, 30 min of extraction time had statistically better extraction yield than 0 and 15 min of extraction time. Even though extraction yield was on the increase as extraction time was on the increase, no statistically significant difference was found between 30 min extraction time and any of more than 30 min extraction time.

Therefore, the method proposed in this study provides a possible pathway to green extraction of bioactive compounds from plant materials, particularly those being accepted as food waste, but having huge potential for industrial applications. Further studies are needed to identify any possible correlation of the parameters used in this study with some antioxidant assays.

Conflict of Interest

The authors declare no conflict of interest.

Ethical statements

The authors declare that this document does not require ethics committee approval or any special permission. Our study does not cause any harm to the environment and does not involve the use of animal or human subjects.

Authors' Contributions

AYDIN C. M.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Visualization, Validation.

GUVEN A.: Validation, Project administration, Software, Review and editing.

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